Effect of *Thyme* Water Extract (0, 1 ml/30 ml buffered rumen fluid) on Short Chain Fatty Acid, Net Energy For Lactation, Metobolizable Energy and Organic Matter Digestibility of Soybean Meal Using In *Vitro Gas* Production Technique

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Abstract: This experiment was conducted to survey effect of adding different levels (0, 1 ml/30ml buffered rumen fluid) of *Thyme water extract* on soybean meal (SBM) degradability were studied by in vitro gas producing techniques. Gas production test with mixtures of filtered rumen liquid of three Taleshi native male cattle rumen in times of 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours were performed. Calculated amounts of organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acid (SCFA) of SM (81.21 g/kg DM, 12.67 MJ/kg DM and 1.24 mmol, respectively) were compared to 0.3 Thyme water extract (70.03 g/kg DM, 10.456 MJ/kg DM, and 0.968 mmol, respectively).
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Abbreviations: SBM, soybean meal; EE, ether extract; ZM, Zataria multiflora; OMD, organic matter digestibility; ME, metabolizable energy, SCFA, short chain fatty acid; CP, crude protein; NE_l, net energy for lactation.

1. Introduction

Ruminal microbial activity is essential for the use of structural carbohydrates and synthesis of high quality protein in ruminants. However, microbial fermentation in the rumen may result in considerable energy and protein losses as methane and ammonia (NRC, 2001; Bunthoeun, 2007).

Methane emission and excretion of N from ruminant livestock substantially implicate global warming and N pollution. Recently, there has been an increased interest in saponins or saponin-containing plants for modifying ruminal fermentation. Saponins are phytochemical compounds composed of a steroid or triterpernoid sapogenin linked to one or more sugar chains (Cheeke, 1999; Bunthoeun, 2007).

Saponins are widely found in plants and are generally grouped among anti-nutritional factors or may be toxic and cause photosensitization (Flaoyen and Wilkins, 1997; Meagher et al., 2001; Pirez et al., 2002; Bunthoeun, 2007). Recently, various findings indicate beneficial effects of saponins to animals and the environment in reducing methane produced by animals (Cheeke, 1996; Takahashi et al., 2000; Pen et al., 2006; Bunthoeun, 2007). Saponins have been shown to react with cholesterol present in membranes of eukaryotic cells but not of prokaryotic cells and therefore, decrease or eliminate protozoa in the rumen without inhibiting bacterial growth (Bunthoeun, 2007).

in vivo, *in situ* and *in vitro* methods have been used to evaluate the nutritive value of feedstuffs. The in vitro gas production technique has proven to be a potentially useful technique to evaluate the nutritive value of feedstuffs, since it gives an estimate of the potential rate and extent of nutrient fermentation in the rumen. However, this technique is measuring gas produced by the fermentation of energy containing components in feeds, and not only that of protein (Mirzaei-Aghsaghali et al., 2008a, 2008b); (Maheri-Sis et al., 2007, 2008); (kiyani et al., 2010).

The objective of this study was to evaluate the potential of natural plant extracts as fermentation pattern *in vitro* gas production characteristics, organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acids (SCFA) and net energy for lactation (NE_l) by *in vitro* gas production technique.

2. Material and Methods

2.1. Thyme Samples

During summer season Thyme samples were collected from different parts of Esfahan province. Next, there were drying for one week, and

homogeneous mixture were papered for nutritive chemical analyzes. For determination of (Thyme) effects, we added Thyme extracts with two levels (0 1 mL: 200 mg samples) into gas test syringes. All samples were then ground in a laboratory mill through a 1 mm screen.

2.2. Procedure of plant extracts preparation

The plant extracts were prepared according to (Patra et al., 2006) with some modifications. The plant materials were dried at 50°C and ground in mills to pass a 1 mm sieve and 100 g placed in 1000 ml of distilled water solvent. The flasks of all the solvents were stoppered and agitated with a magnetic stirrer for 24 h at room temperature. Then the solutions were centrifuged at 3000 g for 10 min. The residue was re-extracted with 500 ml of distilled water for 24 h stirring at room temperature and centrifuged again at 3000 g for 10 min. The plant extracts were combined. Distilled water was evaporated from the solution at approximately 85°C by using a rotary-evaporator (Patra et al., 2006; Sallam et al., 2009).

2.3 Treatments and experimental design

The different levels of thyme water extract were added to the diet sample. Tow levels (0 and 1 ml/30 ml buffered rumen fluid) of thyme water extract were investigated as follow: (i) no additive; (ii) thyme water extract.

2.4. *In vitro* gas production

Fermentation of soybean meal samples were carried out with rumen fluid was obtained from three fistulated Taleshi native male cattle fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). The samples were incubated in the rumen fluid in calibrated glass syringes following the procedures of (Menke and Steingass, 1988) as follows. 200 mg dry weight of the sample was weighed in triplicate into calibrated glass syringes of 100 ml in the absence and presence of level 1 ml (Thyme water extract). The syringes were pre-warmed at 39°C before injecting 30 ml rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39°C.

The syringes were gently shaken 30 min after the start of incubation and every hour for the first 10 h of incubation. Gas production was measured as the volume of gas in the calibrated syringes and was recorded before incubation 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours after incubation. All samples were incubated in triplicate with three syringes containing only rumen fluid-buffer mixture (blank). The net gas productions for soybean meal samples were determined by subtracting the volume of gas produced in the blanks. Cumulative gas production data were fitted to the model of (Ørskov and McDonald 1979).

$$P = a + b (1\text{-}e\text{-}ct)$$

Where P is the gas production at time t, a the gas production from soluble fraction (ml/200mg DM), b the gas production from insoluble fraction (ml/200mg DM), c the gas production rate constant (ml/h), a + b the potential gas production (ml/200mg DM) and t is the incubation time (h).

The metabolizable energy (MJ/kg DM) content of soybean meal was calculated using equations of (McDonald et al., 1995), (Menke and Steingass1988) and (Menke et al. 1979) as follows:

for all feeds,

ME (MJ/kg DM) = 0.016 DOMD

for forage feeds,

ME (MJ/kg DM) = 2.20 +0.136 GP + 0.057 CP +0.0029 CF^2

For concentrate feeds,

ME (MJ/kg DM) = 1.06 +0.157 GP +0.084 CP +0.22 CF -0.081 CA

Where:

GP = The 24 h net gas production (ml/200 mg^{-1}), CP = Crude protein

Short chain fatty acids (SCFA) are calculated using the equation of (Makkar 2005) and (Maheri-Sis 2007, 2008).

Where, Gas is 24 h net gas production (ml/200mg DM).

SCFA (mmol) = 0.0222 × GP – 0.00425

The organic matter digestibility was calculated using equations of (Menke et al. 1979) as follows:

OMD (g/kg DM) = (%)14.88 +0.889 GP +0.45 CP +XA

Where:

GP = About 24 h net gas production (ml /200 mg^{-1})

CP = Crude protein (%)

XA = Ash content (%)

NEL (MJ/kg DM) = 0.115 × GP + 0.0054 × CP + 0.014 × EE - 0.0054 × CA - 0.36 (Abas et al., 2005).

2.5. Statistical Analysis

Data on apparent gas production parameters were subjected to one-way analysis of variance using the analysis of variation model ANOVA of SAS (2000). Multiple comparison tests used Duncan's multiple-range test (1980).

Significance between individual means was identified using the Duncan's multiple range tests. Mean differences were considered significant at ($P<0.05$). Standard errors of means were calculated from the residual mean square in the analysis of variance. All data obtained from three replicates n=3.

3. Results

3.1. *In vitro* gas production

Calculated amounts of organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acid (SCFA) of soybean meal (SBM) are presented in Table 1.

Calculated amounts of organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acid (SCFA) of SM (81.21 g/kg DM, 12.67 MJ/kg DM and 1.24 mmol, respectively) were compared to 0.3 Thyme water extract (70.03 g/kg DM, 10.456 MJ/kg DM, and 0.968 mmol, respectively).

Table 1
In vitro gas production estimated parameters of soybean meal at different incubation times.

Estimated parameters			
	OMD	ME	SCFA
i	81.21	12.67	1.24
ii	70.03	10.456	0.968

OMD: organic matter digestibility (g/kg DM), ME: metabolisable energy (MJ/kg DM), SCFA: short chain fatty acid (mmol)

4. Discussions

There was a positive correlation between NFC content of feeds and gas production, but feed CP, NH_3-N and NDF levels were negatively correlated with gas production (Getachew et al., 2004; Maheri-Sis et al., 2007). Different chemical composition leads to different nutritive value, because chemical composition is one of the most important indices of nutritive value of feeds. Variation in chemical components of feeds such as starch, NFC, OM, CP, NDF and soluble sugars contents can be result in variation of *in vitro* gas production volume (Maheri-Sis et al., 2008).

This study suggested that the thyme water extract have the potential to affect ruminal fermentation efficiency, and be a promising methane mitigating agent.

Salamat azar et al (2011a) evaluation effects of addition three doses zataria multiflora water extract (0, 0.15 and 0.3 ml/30 ml buffered rumen fluid) on the short chain fatty acid, net energy, metobolizable energy and organic matter digestibility of sunflower meal and report the organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acid (SCFA) and net energy for lactation (NEl) contents of sunflower meal were 66.43 g/kg, 8.36 MJ/kg DM, 0.937 mmol and 4.533 MJ/kg DM respectively, while for zataria multiflora water extract (0.3 ml/30ml buffered rumen fluid) were 64.76 g/kg DM, 8.04 MJ/kg DM, 0.895 mmol and 4.664 MJ/kg DM respectively. Salamat azar et al (2011b) evaluation effects of the study three doses zataria multiflora water extract (0, 0.15 and 0.3 ml/30ml buffered rumen fluid) on the short chain fatty acid, net energy, metobolizable energy and organic matter digestibility of canola meal using in vitro gas production technique and report amounts of organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acid (SCFA) and net energy for lactation (NEl) of canola meal (79.46 g/kg DM, 10.27 MJ/kg DM, 1.046 mmol and 5.28 MJ/kg DM, respectively) were high as compared to zataria multiflora water extract (0.3 ml/30 ml buffered rumen fluid) were (41.85 g/kg DM, 3.63 MJ/kg DM, 1.047 mmol and 1.22 MJ/kg DM, respectively). These results are in agreement with the findings of Salamat azar et al (2011a, b).

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